DROUGHT AND HEAT STUDIES
IN ALFALFA

by

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INTRODUCTION

Alfalfa is widely grown because of its adaptability to a wide range of environmental conditions. According to Vavilov (110), alfalfa originated in the Near-Eastern center of crop origin. It was grown by prehistoric man in Mesopotamia (48). Spanish explorers introduced it into the Americas. The acreage planted with alfalfa in the United States in 1960 was 27,368,000 acres, and 67,137,000 tons of alfalfa hay were produced (109). In terms of total acreage and production for all hay crops, those figures constitute 40.9 and 56.9 percent, respectively. In 1960, Kansas planted 1,018,000 acres with alfalfa and produced 2,257,000 tons of alfalfa hay.

The importance of alfalfa in Kansas and as a crop is best explained by Grandfield and Throckmorton (39):

> The importance of alfalfa in Kansas agriculture cannot be overestimated. It has played the role of balancing crop production in two ways; by building soil fertility and by furnishing a high quality feed for livestock. The annual production of alfalfa fluctuates less than does that of most grain crops. Alfalfa is the best of all commonly grown hay crops because it is high in protein and minerals, and is an excellent source of vitamin A.

Alfalfa is a perennial, deep-rooted legume. Alfalfa roots have been traced on an upland soil at the Agronomy Farm at Manhattan, Kansas, to a depth of 25 feet (39). On this same farm, Grandfield and Metzger (38) found that the subsoil moisture was depleted to a depth of 23 feet by alfalfa within two years after seeding. Obviously, after all the subsoil moisture has been exhausted, the crop will depend on the current rainfall. Under drought conditions, alfalfa becomes dormant and this, of course, reduces its yield. That such conditions are not infrequent in Kansas is indicated by the fact that a number of dry periods (ranging between 15 to 50) occurred in the growing season in Kansas during the period 1901-1945, depending on the location in the state (99).

The dry periods in summer are usually accompanied by high temperatures. Kansas summers are best described by Snowden (99):

> Summers are inclined to be warm - often the word "hot" describes them best - but are healthy, with low relative humidity during periods of high temperatures, and usually a good wind movement. Heat prostrations are almost unknown.

The effects of drought and of high temperatures, therefore, cannot be separated from each other under field conditions.

Many factors influence drought and heat resistance of plants. Moreover, weather conditions cannot be predicted over long periods of time. It is preferable, therefore, to study the reactions of plants to drought and heat in the greenhouse and in the laboratory, provided that the methods used give results that are reasonably correlated with field observations.

In addition, breeding for drought and heat resistance necessitates the use of simple laboratory and greenhouse methods that can be used to test and screen large amounts of breeding material.

This investigation was undertaken to determine the reaction of seven alfalfa varieties to various aspects of drought and high temperature damage.

REVIEW OF LITERATURE

Published information on drought and heat resistance of plants has been reviewed thoroughly by Levitt (61). Relation of drought resistance to the physiological processes in plants has been reviewed comprehensively by Iljin (52). Levitt (62) discussed in detail the hardiness of plants to drought, heat, osmotic dehydration and their inter-relationships. Extensive reviews of the work on the physiology of seed germination have been made by Crocker and Barton (23) and by Toole _et al_. (107). In this paper, therefore, only work which is pertinent to this study is included. This review is presented under two headings: simulated drought and heat.

Simulated Drought

Osmotic solutions were found to reduce the rate and total germination of alfalfa seed (108, 82, 28, 46, 83). The reduction, however, was more pronounced in winterhardy than in non-winterhardy varieties (82, 28, 46, 83). Using solutions of NaCl and mannitol, Uhvits (108) concluded that NaCl has toxic effects of alfalfa seeds and seedlings. Rodger _et al_. (82) suggested that germination reduction of alfalfa seed in NaCl and sucrose solutions could be used as a method for determining winterhardiness. Heinrichs (46) germinated seed of 36 alfalfa varieties and strains in solutions of salt and sugar and concluded that the method was unreliable and that the varieties containing a high proportion of _Medicago falcata_ germ plasm have poor germination in solutions of sugar and salt as compared with varieties which are mostly of _Medicago sativa_ origin. Rumbaugh and Swanson (83) germinated alfalfa seeds in mannitol solutions and found that mannitol caused an irreversible inhibition of respiratory metabolism. This effect, however, was not dependent upon the variety or its winterhardiness. These two workers also found that the differences in germination in mannitol between a winterhardy variety (Vernal) and a non-winterhardy variety (Arizona Chilean) were significantly reduced by removal of the seed coat.

Pauli _et al_. (71) found that epicotyl lengths of winter wheat seedlings germinated in mannitol solutions were not significantly correlated with coldhardiness and that this growth response was more closely related to vigor than to winterhardiness.

Wiggans and Gardner (115) studied germination and seedling growth of sorghum and radish in osmotic solutions of glucose, sucrose, mannitol, polyvinylphyrrollidone (PVP, a long chain polymer), and NaCl. They found that PVP and NaCl were toxic to seed germination and that seed germination under high moisture tensions

is at best a test of physiological drought resistance and perhaps then only in seedling stage. Evans (32) found that there was no correlation between growth responses of sorghum seed in mannitol solutions and field observations as to the ability to grow under limited moisture conditions.

McGinnies (66) germinated seed of four wheat grasses, smooth brome, and Russian wildrye in different moisture tensions produced by mannitol and found that increased moisture stress delayed germination and reduced total germination. He concluded that there was no correlation between the drought resistance of these grass species and their germination at high moisture stress. Similarly, Aamodt and Johnston (2) found that the drought-resistant varieties of spring wheat did not have marked superiority over the non-drought-resistant varieties in their ability to germinate in concentrated solutions of NaCl, KCl, and sucrose.

Fryxell (35) reviewed the results of several European workers and noted that selection of different crops for ability to germinate at high osmotic tensions resulted in better yield, quality, and resistance to adverse conditions including drought, salinity, disease, and cold. Bulsonov (15) found that selection of wheat for germination in salt and sugar solutions increased the yield by 20 to 25 percent and that the yield of the selected plants was also higher than that of the controls under humid conditions. He also studied the inheritance of the resulting drought resistance and found it to be transmitted as a constant. Schwer et al. (91) found that the ability of red clover, Ladino clover, and birdsfoot trefoil seed to germinate in mannitol solutions was heritable. Progenies of the selected plants, however, were not significantly different in heat, cold, and drought tolerance from the checks as measured in the greenhouse. They stated that the rate of germination of seeds of different varieties

of alfalfa, white clover, and birdsfoot trefoil indicated that rapid germination under osmotic stress may be related to vigor. Dotzenko and Haus (29) selected alfalfa seedlings from seeds which germinated in mannitol solution having 12 atmospheres of osmotic pressure and found that this ability to germinate was heritable and that the mode of inheritance varied with the variety. Powell and Pfeifer (79) obtained a highly significant correlation coefficient of 0.72 between two generations of winter wheat as to seedling growth rates when the seed were germinated under simulated drought conditions produced by mannitol solutions. Helmerick and Pfeifer (47) stated that varietal differences in the ability of winter wheat to grow and establish stands under limited soil moisture conditions in the field were substantiated by testing the germination and seedling growth in mannitol solutions having osmotic values ranging between 6.5 and 11.5 atmospheres and in soil in which the moisture was reduced before planting by applying 7 or 11 atmospheres of pressure in pressure chambers. They concluded that these varietal responses are inherent since differences due to the location of seed production were not significant.

Rumbaugh and Swanson (83) selected plants from two alfalfa varieties, Vernal, a winterhardy variety, and Arizona Chilean, a non-winterhardy variety, for capacity to germinate under 12-atmosphere tension produced by mannitol. Under both adequate and limited moisture conditions, selection increased dry-matter production in Vernal and decreased it in Arizona Chilean. Their data, however, indicated that selection had no significant effect on the drought resistance of these two varieties as measured by comparing the dry matter production under adequate and limited moisture supplies.

There is a great deal of evidence for a close relationship among resistance to drought, frost, and osmotic dehydration (60, 87, 61, 62). Scarth (86) states

that different kinds and conditions of plant material show parallel variation in their resistance to drought, frost, and plasmolysis. Levitt and Nelson (63) showed that different types of orange peel cells fell into the same order of resistance to drought, heat, frost, and plasmolysis injury. The cells of the most resistant type, however, were larger and had a lower cell sap concentration than the other cells. These observations led Levitt (61) to state that the protoplasmic resistance as measured by resistance to plasmolysis injury is more important than high osmotic pressure and small cell size which are often correlated with resistance to drought, heat, and frost. Irmscher, cited by Levitt (62), found that drought-hardy leafy mosses possess osmotic hardiness. Biebl (7) showed a correlation between resistance to drought and to osmotic dehydration in different ecological types of marine algae. Siminovitch and Briggs (94) found a straight line relation between critical dehydration intensities produced by desiccation and osmotic dehydration of the living bark tissue of the black locust tree. They also found a straight line relation between critical dehydration intensities produced by desiccation and frost in this same tissue. Bartetzko, cited by Levitt (62), showed that the frost killing temperature of Aspergillus niger decreased with the increase in the concentration of dextrose used in the culture media, indicating possible osmotic hardening. Kylin, cited by Levitt (62), found correlation between the frost killing temperature and the osmotic hardiness of several species of algae.

Milthorpe (68) reported that there was no correlation between the resistance of seedlings of two wheat varieties to desiccation over concentrated sulfuric acid and the known drought resistance of the two varieties.

According to the mechanical theory of drought injury advanced by Iljin (52), the death of cells results from the mechanical injuries which accompany their

desiccation and remoistening. In a study of the mechanism of frost injury to plants, Siminovitch and Scarth (96) found that death of cells may result from rupture of the protoplasm upon deplasmolysis. Scarth (86) stated that the maximum plasmolysis that cells can withstand, when exposed to drought, frost, or osmotic dehydration, is determined by the point at which an irreversible stiffening, presumably coagulation, of the ectoplasm occurs and that the immediate cause of death is the rupture of the rigid ectoplasm upon deplasmolysis. Siminovitch and Levitt (95) found a correlation between frost hardiness and resistance to injury by deplasmolysis in two winterhardy and two non-winterhardy varieties of alfalfa. Scarth and Levitt (88) showed that resistance to injury by deplasmolysis is greater in frost-hardened than in non-frost-hardened cells of different kinds of plant material. Siminovitch and Briggs (93) and Briggs and Siminovitch (13) used the resistance of the living bark tissue of the black locust tree to injury by plasmolysis and deplasmolysis as a direct measure of the frost resistance of this tissue and the results obtained were in agreement with the expected seasonal course of frost hardening. Whiteside (113) found that the desiccation resistance of cells increased with both drought and low temperature hardening of wheat and that the desiccated cells hardened by low temperatures had greater injury upon remoistening when they were not allowed to imbibe the water slowly.

This mass of evidence points to a close correlation between the physiological resistance of plants to drought, osmotic dehydration, and deplasmolysis injury and that osmotic solutions can be used to test the physiological drought resistance of plants and their ability to harden. Thimann (106) showed that growth (elongation) of pea stems decreased, over a wide range of mannitol concentrations, in linear proportionality to the logarithm of the concentration. He also found that mannitol did not interfere with the metabolic processes and that the sections resumed normal growth immediately after a period in mannitol.

He concluded that mannitol is the best substance to prevent water uptake osmotically. Hayward and Spurr (44) found that the entry of water into corn roots was reduced to about the same level in a solution of mannitol, sucrose, Na_2SO_4, NaCl, or $CaCl_2$ having an osmotic pressure of 4.8 atmospheres. The uptake of water, however, was less from the mannitol solution than from the other solutions when the osmotic pressure of the solution was 2.8 atmospheres. The water intake was measured by potometers and the measurements were made over relatively short periods (5.5 - 6 hours). The extensive evidence of specific toxic effects of relatively high concentrations of ions has been reviewed by Hayward and Wadleigh (45). Collander and Burland, cited by Hayward and Spurr (44), found that cells of Chara ceratophylla did not take up appreciable amounts of sucrose, glucose or mannitol in 48 hours.

Gingrich and Russell (36) found differences in the growth responses of small corn seedlings when grown for 24 hours in soil and mannitol solutions having equal moisture stresses. They attributed these differences to differences in the water-transmitting characteristics of the two media. However, it is well-established that the greater part of the water requirement of plants is secured from the soil through root extension rather than water transmission (67). Dittmer (27), for example, showed that a 4-month old rye plant produced 114,179 new roots, totaling 3.1 miles in length, per day and 118,475,737 new root hairs, having a total length of 55 miles, per day. In fact, for this reason, the growth characteristics of the roots of the variety or species are generally considered as being of importance in the drought resistance of that variety or species. These two factors, soil characteristics and growth characteristics of the roots, are two major complicating factors in studying drought resistance, especially under field

conditions. Since such complicating factors do not exist in osmotic solutions, this suggests the use of osmotic solutions in testing relative physiological drought resistance.

Burton et al. (16) used the dry matter production of grasses in a dry year as an index of drought tolerance and found reasonable correlation between this index and symptoms of drought injury (wilting and firing). Further, they showed that root yields did not correlate with the drought tolerance of the grasses studied. In a later study by Burton and co-workers (17), it was found that firing due to drought, dry-matter production in a dry year, water use, and wet year to dry year ratios in dry-matter yield and water use were significantly related for the five grasses studied and that the best differentiation between those grasses was obtained under severe drought conditions.

Dry-matter yields, as well as mineral yields, of forage crops are of practical importance to the farmer, especially in dry years. Wadleigh and Richards (111) and Richards and Wadleigh (80) reviewed much of the work on the mineral nutrition of plants under soil moisture stress. They stated that most experimental evidence showed that decrease in available soil moisture is associated with a definite increase in the nitrogen content of the plants, a definite decrease in the potassium content, and variable effects on the contents of phosphorus, calcium, and magnesium. In this same review (80) they also stated that most evidence indicates that growing plants under drier soil moisture regimes results in a high percentage composition of the various mineral elements.

Daniel and Harper (25) reported that with a decrease in effective rainfall, there was a decrease in phosphorus and an increase in calcium contents of alfalfa. In a more recent study, similar results were obtained with

alfalfa over a period of ten years by Harper (42). Leamer, Olsen, Larson, and Domingo[1/] found that nitrogen content of alfalfa decreased with the decrease in the number of irrigations. Jenne et al. (53) found that the yields of phosphorus, dry matter, nitrogen, magnesium, potassium, and calcium from mature corn plants grown with decreasing soil moisture supply were 40, 44, 50, 65, 71, and 93 percent, respectively, of the yields of mature corn plants grown under conditions of adequate moisture. These yields, obviously, indicate a decrease in phosphorus content, and an increase in the contents of nitrogen, magnesium, potassium, and calcium upon the decrease in moisture supply. Woodman et al. (117) found higher calcium and lower phosphorus contents of pasture grasses during the period of low rainfall than during the period of high rainfall in the same growing season. In a similar study by Ferguson (33), it was shown that during the dry period of the growing season there was a decrease in the nitrogen and phosphorus contents, a slight decrease in the calcium content, no change in the potassium and sodium contents, and a definite increase in the chlorine content. During the rainy period of the growing season, however, there was an increase in the nitrogen, phosphorus, and sodium contents, and a decrease in the calcium, potassium, and chlorine contents. In a recent study on the effect of moisture supply on the mineral uptake of eight forages including two alfalfa varieties, Kilmer et al. (57) concluded that the general over-all effect of increasing soil moisture availability was to increase the yields of nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur. The increased mineral yield, however, was mostly due to the increase in top growth.

[1/] Personal communication with Dr. C. A. Larson.

It is obvious, from the review by Richards and Wadleigh (80), and from the few papers mentioned above, that the results from studies on the mineral uptake of plants as affected by moisture stress are most conflicting. The great number of environmental factors involved in the availability of mineral nutrients in the soil, the ability of the plants to absorb and utilize these mineral elements, and the complex effects of each of these factors on the others, would make any generalization regarding this problem inappropriate. Obviously, the complicating factors due to the chemical, physical, and microbiological characteristics of soils, and differences in root penetration, distribution, and activity, can be eliminated by growing the plants in culture solutions to which a suitable osmotically-active substance has been added. The information obtained by the use of this technique, however, would be pertinent only to the effects of the moisture tension *per se* on the mineral yields of plants.

The extensive work on the gibberellins has been thoroughly covered by several reviews (103, 9, 11, 102, 116, 104, 10, 105, 5). Phinney and West (77) reviewed the evidence that the gibberellins are native plant growth regulators. Growth responses of plants to gibberellins are often at maximum when growth is adversely affected by environmental or certain genetical factors. Pauli and Sorensen (72) reported that treating dwarf alfalfa plants with gibberellic acid induced elongation, but not flowering, of these plants. Brian and Hemming (12) stated that the response of dwarf and tall varieties of pea to gibberellic acid was linearly and inversely related to the logarithm of growth rate at the time of treatment. Allan *et al.* (3) found that seed treatment with gibberellic acid produced the greatest stimulation of emergence in the slow-emerging varieties of wheat and that the rapid-emerging varieties

showed little or no response, and sometimes were adversely affected by the treatment. Evans (32) reported that gibberellic acid increased the rate and percentage of germination of sorghum seed germinated under high moisture tension (15 atmospheres) produced by mannitol. He also found that gibberellic acid caused an increase, which varied at the different moisture tensions, in plumule growth but had no significant effect on radicle growth.

Hayashi (43) reported that gibberellic acid stimulated germination of barley, wheat, and rice seed. Pauli and Stickler (73) found that treatment of sorghum seed with gibberellic acid increased the rate of germination and emergence, but had no effect on final germination or emergence. They also found that maturity, plant height, grain yields and yield components were not influenced by seed treatment or foliar spray at bloom time. Repeated foliar sprays during vegetative growth caused increases in height and yields of grain and grain plus forage, but these increases were not consistent. Stickler and Pauli (101) reported that treatment of winter wheat seed with gibberellic acid reduced seedling emergence, vigor, and regrowth after cutting, and forage and grain yields. Pauli and Stickler (74) found that the effects of gibberellic acid on winter wheat varied with the formulation and method of application. Pauli, et al. (75) showed that gibberellic acid did not replace the cold treatment required for winter wheat vernalization but it had a supplemental effect only when the minimum cold treatment was given. Finn and Nielsen (34) reported that foliage applications of gibberellic acid to six grass and legume species, including alfalfa, increased the dry-matter yields of the first cutting but reduced those of the second cutting. Corns (22) reported that foliar applications of gibberellic acid increased elongation of stems and leaves of Kentucky bluegrass and Kharkov winter wheat but not of alfalfa and

that the treatment increased the fresh and dry weights of Kentucky bluegrass, increased the yield of Kharkov winter wheat, and had no effect of the dry weight of alfalfa.

In an extensive study on the effects of gibberellic acid on different plants, Marth _et al._ (64) concluded that stem elongation was the most obvious response and that rapid and prolonged stem elongation of the treated plants was associated with less root growth. The importance of the growth of roots in supplying the moisture requirements of the plants has already been pointed out above. It would appear then, that the inhibitory effect of gibberellic acid on root growth would be disadvantageous under dry conditions.

Mueller and Weaver (69) made mixed plantings of 14 species of prairie grasses in tubs of soil and the seedlings were exposed to soil drought until the tops became dry and many leaves felt brittle. They found good agreement between the ability of the different grass species to survive this experimental soil drought and their expected resistance to natural drought based on their ecological distribution. McAlister (65) grew grass seedlings in soil flats and allowed them to wilt until a definite, calculated soil moisture was reached to determine their resistance to soil drought as measured by survival. The method gave extremely variable, and therefore unreliable, results and another procedure was used. The flats were allowed to dry out in a drought chamber until all the leaves became dry and brittle. The survival of the grass seedlings after this treatment was in agreement with the expected resistance of the mature plants of the different species and of the different strains within each species studied. Differentiation among strains of the same species, however, was satisfactory when the seedlings were six weeks or two months old, but not when they were one month old. Platt and

Darroch (78) studied the resistance of a large number of wheat varieties to soil drought by growing them in one-gallon crocks and allowing them to deplete the soil moisture and reach permanent wilting. The plants were allowed to remain in this state for definite periods of time after which they were watered and noted for survival. Varietal differences were found to exist and were relatively consistent.

Heat

Went (112) and Hagan (40) have reviewed the work on the effects of air and soil temperatures on germination and subsequent growth of plants. Schonhorst _et al_. (89) studied the growth response of nine varieties of alfalfa to all possible combinations of three constant temperatures (60°, 70°, and 80° F.) and three daylengths (8, 12, and 16 hours). The greatest stem elongation was noted at a temperature of 70° F. and a daylength of 16 hours. Gist and Mott (37) studied the response of alfalfa, red clover, and birdsfoot trefoil seedlings to all combinations of four constant temperatures (60°, 70°, 80°, and 90° F.), three light intensities (200, 600, and 1200 foot-candle) for 12 hours, and four levels of soil moisture. They found a decrease in the growth (dry weight) of these legumes with the increase in temperature and increase in light intensity. It is difficult, however, to evaluate the results of these two workers because of the relatively high night temperatures associated with day temperatures of 80° and 90° F. Jones and Tisdale (54) grew seedlings of alfalfa and some other legumes in metal cans with soil temperatures ranging between 53.6° and 96.8° F. while the air temperatures were 57.2° to 68° F. Data on the amount of growth made by the alfalfa seedlings were not given but pictures showed that alfalfa seedlings grew even when the soil

temperature was as high as 96.8° F., but best top growth occurred at a soil temperature of 69.8° F.

Hide (50) reported that during a period of hot clear summer weather, July 21 to 24, 1941, at Manhattan, Kansas, the maximum soil temperatures at a depth of 1/4 inch in a fallowed silty clay loam soil ranged between 144° F. and 156° F. while the maximum air temperatures ranged between 100° and 104° F. Generally there was a daily period of about 11 hours during which temperatures of 110° F. or more occurred in the soil at a depth of 1/4 inch. Other workers (8, 97) also reported soil temperatures that were about 30° F. higher than air temperatures. Such extremely high temperatures, however, generally occur only for relatively short periods.

Edwards (31) reviewed most of the early work on the temperature relations of seed germination. Coffman (21) reported that seed of Kansas Common alfalfa germinated best at a temperature of 50° to 60° F. Many studies have been made on the effects of high temperatures on the germination and emergence of many plant species and the different species were found to vary widely in their reactions to this factor (100, 59). Further, different seed lots of the same sorghum variety were found to vary in their emergence at different temperatures (32).

The work on the effects of air and soil temperatures on the mineral nutrition of plants has been reviewed thoroughly (40). A great number of factors are involved in the effects of temperature on the nitrogen content of plants. There is evidence, however, that the nitrogen content of plants increases with the increase in temperature. Brown (14), for example, showed that there was an increase in the nitrogen content of Kentucky bluegrass, orchard grass and Bermuda grass with the increase in temperature above the optimum for growth.

Dry-heat treatment of alfalfa seeds is known to increase their germination percentage by increasing the permeability of the hard seeds. Rinker (81) found that such treatments did not produce any visible abnormalities in the resulting plants. Some Russian workers claimed that certain pre-sowing treatments of seed can be used to increase the drought resistance of the resulting plants (114). These treatments include the method of heating the seeds at 176° F. for four hours. However, there did not seem to be any reference in the literature to the use of the dry-heat treatment of seed as a method to measure the relative physiological drought resistance of plants.

Several methods have been used to test the resistance of plants to moderately high or high temperatures associated with low relative humidities and availability of soil moisture. The methods included the use of heat chambers or rooms (51, 49, 20, 84), artificial drought chambers (1, 2, 90, 56, 26), draft ovens (18, 19), and artificial hot winds (6, 69). In general, the results showed correlation with the drought resistance of the tested species and varieties as determined by field observations (1, 2, 51, 6, 90, 69, 18, 84). However, Chen (20) did not find correlation between the resistance to artificial heat in heat chambers and resistance to artificial soil drought in the sorghums and Kaoliangs which he studied. Heyne (49) noted close agreement in heat chamber and field resistance for most inbred lines of corn evaluated. Both Heyne (49) and Chen (20) tested the plants for heat resistance in the seedling stage. Carroll (19) found that the order of resistance to drought was the opposite of the order of resistance to heat for a minority of the grass species which he studied. Kenway _et al_. (56) tested the resistance of a large number of spring wheat varieties to atmospheric drought in an artificial drought chamber for four to 18 hours. The tests were run at a

temperature of 110° F., a relative humidity of 14-23%, an air velocity of 10 mph, and the pots were watered before and after the treatment. Varietal differences were found to exist but were not consistent in different tests.

High temperatures injure plants by direct thermal injury, dehydration, and some other indirect effects. The relative humidity associated with the high temperature, therefore, is an important factor in artificial heat tests. This factor is important even when the plants are well supplied with moisture during the test due to its effects on the transpiration rate, and therefore on the cooling effect of transpiration. Levitt (61, 62), therefore, states that the correlation between the resistance of plants to high temperatures associated with low humidities and their resistance to drought in the field is due to the possibility that these heat tests have actually measured the resistance of plants to desiccation. The direct thermal effect of high temperatures on the protoplasm, however, is completely independent of dehydration injury (62).

Julander (55) found close correlation between the heat resistance of the rhizomes of four grass species and the aridity of their natural habitats. He pointed out that survival of grasses under hot and dry conditions may be determined in part by the resistance of their crowns and underground parts to high soil temperatures.

Diurnal variation in heat resistance has been reported for several plants including alfalfa (49, 58, 56). The plants were found to be most susceptible to heat when tested early in the morning, and exposure to light was shown to be the factor involved. Laude (58) reported that plants reached their daily maximum resistance to heat within 4 hours after exposure to daylight.

MATERIAL AND METHODS

Information pertaining to the seed lots of the seven alfalfa varieties used in this investigation is presented in Table 1. The same seed lots were used throughout the investigation.

Table 1. Identification number, source, and date on which the seed lots were received by the Crops Research Division, A.R.S., U.S.D.A., of the seven alfalfa varieties.

Variety	F. C. No.	Source	Date received
Teton	34,112	South Dakota	1957
Buffalo	33,557	California	Sept. 1956
Ladak	32,566	Canada	Feb. 1955
Rhizoma	34,035	Canada	Feb. 1958
Rambler	34,135	Canada	March 1958
African	33,602	Arizona	Nov. 1956
Nomad	34,054	Oregon	Feb. 1958

Laboratory germination tests indicated high germination percentages of the seed lots.

Unless otherwise stated, the following methods and materials were employed throughout the study. Germination experiments were carried out in 9 cm. Petri dishes. Two 9 cm. filter papers were placed at the bottom of the dish and 5 ml. of the desired solution or of tap water were used to saturate them. The seed were then placed and covered by another filter paper. The dishes were placed in a growth chamber, or a seed germinator, adjusted to a constant temperature of $70^{o} \pm 2^{o}F$. Germination proceeded in the dark and the seed was considered to have germinated when the radicle reached 5 mm. in length.

Final germination percentages were taken seven days after the day on which the tests were started. This procedure is in accordance with the official germination test for alfalfa seed.

The amounts of mannitol, $CH_2OH(CHOH)_4CH_2OH$, required to produce the desired osmotic tension were calculated from the following formula given by Helmerick and Pfeifer (47):

$$P = \frac{gRT}{mV}, \text{ where:}$$

P = osmotic pressure in atmospheres
g = grams of mannitol
R = 0.08205 liter atmosphere per degree per mole
T = absolute temperature
m = molecular weight of mannitol
V = volume in liters

The metal flats and the pots used were 14" x 20" x 3 3/4" galvanized metal flats and 4-inch unglazed clay pots, respectively, both of which had drainage holes. The soil was a silty clay loam from the Agronomy Farm at Manhattan, Kansas, mixed with sand and sheep manure in a 3-1-1 ratio, respectively. The pH of the mixed soil was 7.2. River sand and vermiculite were used in some experiments as indicated later. All the flats, pots, soil and sand used were sterilized in the steam sterilizer at a pressure of 15 lbs. for 4 hours. Immediately before planting the seed, the proper rhizobium inoculant was sprinkled on the soil, sand or vermiculite, and then the seed placed and covered.

Whenever border rows were planted, seeds of the alfalfa variety Cody were used. The growth chambers used were 2 1/2 x 4 x 5 feet with automatic temperature control. Light was supplied to the plants growing in each chamber by 12 twenty-watt white fluorescent tubes and 4 two-hundred-watt white incandescent bulbs. A fan circulated the air in the chamber. The heat room had internal

dimensions of 5 1/2 x 6 x 7 1/2 feet with a turntable in the middle. Hot air of controlled temperature circulated in the room and light was supplied to the plants during the test by 3 two-hundred-watt white incandescent bulbs placed 1 1/2 foot above the plants.

Drying of plant material for dry-weight data and chemical analysis was done in an air-draft oven at $70^{\circ} \pm 1^{\circ}$C. for 24 hours. The Gunning modification of the Kjeldahl method (4) using boric acid in the receiving flask (85) was followed in making the analysis for total nitrogen. Analyses for potassium and phosphorus were made by the Chemical Service Laboratory of the Biochemistry Department, Kansas State University.

Nutrient elements were supplied to the plants grown in the greenhouse or growth chambers by watering them at planting time and each two to three weeks thereafter by a solution of Hyponex having the recommended concentration (5 gms. of Hyponex per gallon of water). The Hyponex used contained the following percentages of water-soluble nutrients: 7% nitrogen, 6% phosphoric acid, and 19% potash. Whenever it was necessary, suitable insecticides were used at the recommended rates for control of insects in the greenhouse.

The specific materials and methods employed in each experiment will be described in the following sections.

Germination and Seedling Growth in Mannitol

Four 100-seed lots from each of the seven varieties were germinated in each of four moisture tensions (0,4,8 and 12 atmospheres) in a completely randomized design. The seed lots germinated in tap water (0 atmosphere tension) served as checks. Mannitol solutions of the required concentrations provided the other moisture stresses.

Daily germination counts were made during a period of one week and on the eighth day the length of the elongated part (radicle and hypocotyl) of each of ten seedlings, taken at random from each Petri dish, was measured.

Germination and Seedling Growth in Mannitol and Gibberellic Acid

Only three varieties: Teton, Buffalo, and African were used in this experiment. Three 100-seed lots of each of these three varieties were germinated under all possible combinations of four stresses (0, 4, 8 and 12 atmospheres) and four gibberellic acid concentrations (0, 50, 100 and 150 ppm.) in a completely randomized design. The required amounts of gibberellic acid were weighed and dissolved in tap water or mannitol solutions having the desired moisture tensions. The seed lots germinated in tap water (0 atmosphere moisture stress and 0 ppm. of gibberellic acid) served as checks.

The data for the rate of germination, final germination percentage and seedling growth were collected in this experiment in a similar manner to that of the preceding experiment.

Hardening in Mannitol

Three 100-seed lots from each of the seven varieties were germinated in 0 and 6 atmosphere tensions in a completely randomized design. The study was to be conducted with normally-developed, rather than etiolated, seedlings. Therefore, the seeds were not covered by a filter paper in the Petri dish and a 16 hour photoperiod was maintained. These lights supplied an intensity of about 700 foot-candles a few inches above the transparent glass covers of the Petri dishes. The seedlings did not show symptoms of etiolation and

normal green color developed in the cotyledons. However, it was evident as soon as germination started that the "6" seedlings were somewhat delayed in germination and reduced in size. To compensate for these differences in rate of development and size, two more 100-seed lots from each variety were germinated in tap water in the same growth chamber three days after the beginning of the experiment. The "0" seedlings were considered unhardened and the "6" seedlings hardened. One hundred seedlings each from 0 and 6 atmospheres of about the same size and stage of development were taken at random from the respective Petri dishes of each variety one week after the beginning of the experiment. They were then placed in a mannitol solution having a moisture stress of 16 atmospheres. The test was carried out in two 12 1/2" x 9" x 3/4" plastic trays. Two layers of white paper towels were placed at the bottom of each tray and 100 ml of the test-solution were used to saturate the towels and provide a thin layer of the free solution on their surface. Each tray was divided by lines into nine equal compartments. Each 100-seedling sample was placed in a compartment and two 10-seedling sub-samples were removed from it at random after each of five test periods (4, 8, 12, 16 and 20 hours). The seedlings were partially immersed in the test solution during the test. The trays were placed throughout the test in the growth chamber at $70^{\circ} \pm 2^{\circ}$F. and with the lights turned on. The trays used were the lids of cake pans which provided a tight cover for the trays during the test. The relatively low temperature in the growth chamber and the covering of the trays minimized evaporation from the test solution. The seedlings were exposed to light frequently at the times during which the sub-samples were taken out.

Upon removal from the high tension solution, seedlings of each sub-sample were placed separately and gently in a large strainer and were thoroughly washed free of mannitol.

Three 9 cm. filter papers were placed in the bottom of each of 35 Petri dishes and were saturated with 5 ml. of tap water. Each Petri dish was divided into four equal compartments. Each sub-sample was then placed in a compartment and the seedlings left to recover for five days in the growth chamber at a constant temperature of $70^{\circ} \pm 2^{\circ}$F. and 16 hours of light each day. After this recovery period, the surviving seedlings were counted. The hypocotyls of the living seedlings showed negative geotropism which was not observed in the dead seedlings.

Growth in Mannitol-Nutrient Solution

This experiment was conducted in a cooled greenhouse in July where the mean temperature was approximately 81.8°F. The temperature and relative humidity in the greenhouse were recorded by a recording hygrothermograph.

Twelve 30 1/2" x 14 1/2" x 7/8" galvanized metal trays were filled with coarse vermiculite which was then saturated with Hyponex solution. A 31 1/2" x 14 3/4" x 3/4" tray, the bottom of which was 8-mesh galvanized screen, was fitted on the top of each vermiculite tray. Sand was placed evenly in a 1/8" layer on the screen. Sixteen 12"-long rows, equally spaced (1 3/4") were marked in the sand layer. The two outer rows served as borders. Counted seed lots from each of the seven varieties were sown at random in the first seven rows following the border and the varieties were rerandomized in the next seven rows. The number of seed per row varied with the variety and was calculated, from the germination data in sand of a previous experiment, to produce, on the average, fifty plants per row. The seed were equally spaced in the rows and after planting all the rows and borders, a 1/4" layer of sand was placed evenly over the first layer to cover the seed.

The same procedure was followed in planting each of the twelve trays. The high moisture-holding capacity of the vermiculite kept the sand layer moist during the period of germination. Later, water was supplied to the vermiculite from under the screen tray to keep the sand from being washed down. Perfect stands were obtained and the plants made excellent growth.

Twelve 30" x 14" x 5" galvanized metal tanks were placed on a level bench in the greenhouse and 28 liters of tap water were placed in each tank to bring the level of the water in the tank to about one inch from the top and this level was marked. About 56 gms. of Hyponex and 2 gms. of Versenol iron chelate were dissolved in each tank. Continuous aeration of the solution was attained by connecting a copper pipe placed in the solution to the compressed-air supply. Each screen tray with the plants growing in it was then removed from the vermiculite tray and fitted on the top of a tank chosen at random. The plants were 18 days old and were well supported in the screen by their stems at the time of transfer. The roots pulled easily from the vermiculite flats and were intact with some of the vermiculite attached to them. The roots were submerged in the nutrient solution when the tray was placed on the tank and tap water was added daily to bring the level of the solution up to the mark. All the trays and tanks had similar length and width. Therefore, the trays provided opaque covers for the tanks and the roots grew in the dark.

The plants were grown in the tanks for eight days before the addition of the mannitol. The 12 tanks were situated in three replications, and four moisture tensions (0, 4, 8 and 12 atmospheres) were assigned at random to the four tanks in each group. The contribution to osmotic pressure of the salts in the nutrient solution was considered negligible and the required amounts of

mannitol were dissolved in the nutrient solution when the plants were 26 days old. The same moisture stress was maintained throughout the test by frequent additions of tap water to maintain the solution at a predetermined level.

During the test it became evident that the plants took up some of the mannitol and after five days it was noticed that the presence of mannitol in the nutrient solution favored the growth of some saprophytic fungi. Phygon was applied at a rate of 1/2 teaspoonful (about 2 gms.) per tank to all the tanks in an effort to control the fungi. The growth of the fungi, however, was only partially checked by the fungicide. The fungicide did not have any visible toxic effect on the plants, as indicated by the healthy appearance of the check plants. The uptake of mannitol and the growth of the fungi did not produce any symptoms of toxicity for plants growing in the mannitol solutions as evidenced by the appearance of the plants in the 4-atmosphere tanks and by the initiation of new growth from the crowns of the plants growing in the 8- and 12-atmosphere tanks after the severe burning of the tops caused by the high moisture stresses. These two interferences, however, proved to limit the usefulness of the method.

The test was terminated after ten days when the plants were 36 days old. The check plants (0 atmosphere tension) were in good growth condition at the end of the experiment. The plants in all the tanks did not show any symptom of a disease or mineral deficiency throughout the experiment.

The roots in each row were tangled with the roots of the adjacent rows and therefore only the tops of the plants were harvested. The tops in each row were harvested, dried, and weighed separately. However, the proper statistical analysis of the dry-weight data necessitated the summation of the dry weights of the tops from the two rows of the same variety in the same

tank together. The relative dry-matter yield under moisture stress was considered the most reliable index of drought resistance in this experiment.

Relatively small amounts of dry matter were obtained from each row and therefore the dry tops of the six rows of the same variety and tension were composited together to produce the samples used for chemical analysis. The composited samples were ground by running them first through a big Wiley grinder, and second through a small Wiley grinder using a 30-mesh screen. Duplicate one-gram samples of the ground material were analyzed for total nitrogen by the Kjeldahl method. The remaining material was sent to the Chemical Service Laboratory to be analyzed for phosphorus and potassium.

Drought Tests in Flats

Metal flats were partially filled with sand and were then saturated with Hyponex solution. Seven 1 3/4-inch rows and four border rows were marked and planted in a similar manner to that of the previous experiment except that a 1/2" layer of sand was used to cover the seed. The outward side of each flat was bordered with a row of Cody alfalfa. Seven flats were planted in a modified complete block design. The seven varieties were assigned to the seven rows of each flat in a predetermined arrangement so that each variety occurred in all the seven possible positions. The purpose of this arrangement was to minimize any possible position effect. The varieties were planted at random in another flat which served as a check. The flats were placed in a level position on the same bench in the greenhouse.

Six days after planting, the seedlings were thinned to the same number of seedlings per row, and watering was withheld from the seven flats to be tested when the seedlings were in the first trifoliolate stage (13 days old).

In an effort to expose the different parts of each flat to about the same environmental conditions, the flats were rotated frequently when the sand started to dry. Each flat was rewatered separately when the plants permanently wilted and the leaves felt dry. The period of the test was about six days under normal greenhouse conditions in June. The exact time of rewatering, however, varied with the different flats. A check flat was kept under optimum moisture conditions throughout the experiment.

The surviving plants were left to recover for a period of 11 days under optimum moisture conditions. At the end of the recovery period, the surviving plants were counted and the survival percentage was considered to be the suitable index of drought resistance in this experiment. The check plants grew well throughout the experiment.

Essentially the same procedure was used to plant and test the varieties in the second trifoliolate stage (16-day old seedlings). A similar set of sand flats was also planted and the plants were tested for the first time when they were two months old and the surviving plants were retested at the age of three months. The length of the testing and recovery periods varied with the different tests and whenever the plants were grown for any considerable length of time in the flats, Hyponex and Versenol iron chelate (0.1 gm. per gallon of Hyponex solution) were used to keep the plants under optimum mineral nutrition.

Another set of flats was planted in practically the same manner described above except that plaster-grade vermiculite was used instead of sand. The vermiculite was saturated with Hyponex solution and mixed thoroughly before placing it into the flats. The plants made good growth and water was withheld when plants were 25 days old. The same method was used to plant another set of vermiculite flats except that no water was given to the flats after the initial mixing and watering of the vermiculite with Hyponex solution. Under normal greenhouse conditions in March, the plants started to wilt at the age of 24 days.

Emergence and Growth Under Different Temperatures

Three growth chambers were set to three temperatures, $70^\circ \pm 2^\circ$, $90^\circ \pm 2^\circ$ and $110^\circ \pm 2^\circ$F. during a 16-hour photoperiod. The temperature during the 8-hour dark period was $70^\circ \pm 2^\circ$F. in each chamber. A similar procedure to that described for planting the sand flats was followed in planting 9 soil flats except that the number of seeds per row was 60 for each variety and the seven varieties were assigned at random to the rows in each flat. Three flats at random were placed in each chamber and the three chambers were considered three complete blocks of a modified complete block design. All the flats were rotated and changed in position within the respective chambers every day to expose the flats within each chamber to about the same growth conditions. Growth proceeded uniformly in the three flats of each chamber and the flats in the 70° - 70° F. chamber served as checks.

Daily counts of emergence were made during a two-week period after planting. During the last few days of that period only few seedlings emerged and therefore the counts on the fourteenth day were considered the final emergence. The seedlings were then thinned to 24 seedlings per row in the flats growing under 70° - 70° and 90° - 70°F. Emergence was very poor in the flats growing under 110° - 70°F. and therefore the seedlings were not thinned. The average height of the plants in each row was measured each three days during the period between the twelfth day after planting and the end of the experiment.

The tops of plants in each row were harvested separately when they were 27 days old and were dried and weighed. The plants growing under 110° - 70°F. made very little growth and therefore only the tops of the plants grown under 70° - 70° and 90° - 70°F. were used for total nitrogen analysis. The tops were

ground in a small Wiley grinder using a 20-mesh screen and the analysis for total nitrogen was run on duplicate 0.1 gm. samples in a micro-Kjeldahl apparatus.

The growth chambers had no device for humidity control and therefore the relative humidity in the chambers varied with the temperature. Representative temperature and relative humidity measurements were recorded for each growth chamber during the experiment by a recording hygrothermograph. The temperatures did not vary beyond the limits stated above, except at short periods of time when it was necessary to open the doors of the chambers. The relative humidity, however varied between 35 and 60, 48 and 56, and 50 and 74 percent in the 70°- 70°, 90°- 70°, and 110°- 70°F. chambers respectively, during the photoperiod. During the dark period, the relative humidity varied between 85 and 100 percent in the three chambers.

High-Temperature Tests

Sets of pots were used in these tests. Each set consisted of three pots for each of the seven varieties in a completely randomized design. The same method was used to plant all the sets. Each pot was partially filled with soil which was then saturated with Hyponex solution, 25 seeds were sown, and were covered evenly by a cupful of soil which provided a 3/8" layer of soil over the seed. After emergence, the seedlings were thinned to 15 seedlings per pot.

Two sets of pots were tested separately in the heat room for 10 and 14 hours when the plants were 32 and 34 days old, respectively. The temperature in the heat room varied between 127° and 133°F. with a relative humidity of 23-24 percent. Preliminary tests indicated that the soil in the pots dried

out after about 3 hours in the heat room and therefore the pots were watered after each two hours during the period of the tests to prevent any soil moisture loss.

Relatively high percentages of the top tissue showed severe firing after the tests but none of the plants were killed. The plants were left to recover for three days in the greenhouse and at the end of the recovery period, the pots were rated according to the percentage of top-tissue killed.

Another set of pots was tested in the heat room for 14 hours when the age of the plants was 43 days. The temperature and relative humidity during the test were 126° - 133°F., and 21 - 27 percent, respectively. At that age of the plants, however, the test caused only slight firing of the tops and therefore, after a recovery period of 15 days, the plants were subjected to another test in the growth chamber when they were 58 days old. The temperature during the six hours of the test was 130° ± 2°F. and the relative humidity 70-83 percent. The tops of the plants were severely fired by the test and some plants were killed. The surviving plants were counted after a recovery period of two weeks.

Two other sets of pots were tested separately for 14 hours in the heat room when the seedlings were in the first trifoliolate stage (14 days old). One of the tests was at a temperature of 126° - 132°F. and a relative humidity of 28 - 29 percent, and the other test was at a 125°-131°F. temperature and a 30-32 percent relative humidity. Some of the seedlings were killed and the surviving seedlings were counted after a recovery period of two weeks.

The plants of the first three sets of pots mentioned above were planted and grown under normal greenhouse conditions during May, June and July. The seedlings of the other two sets of pots were planted and grown during July and August in the cooled greenhouse mentioned earlier. All the pots were

placed on a metal surface to prevent any tearing of the roots upon the removal of the pots, which were to be tested, from the bench.

Representative soil temperature measurements at a depth of one inch in the center of the pot showed that the maximum soil temperature was 120°F. in the growth chamber and ranged between 112° and 114°F. in the heat room.

The tests in the heat room were started at 9:00 or 9:30 a.m. and the test in the growth chamber was started at 1:30 p.m. The lights of both the heat room and the growth chamber were turned on throughout all the tests.

Dry-Heat Treatment of Seed

Three 100-seed lots of each of the seven varieties were treated in an air-draft oven under all possible combination of two temperatures (176° ± 2° and 194° ± 2°F.) and four durations (30, 60, 90 and 120 minutes) in a completely randomized design. Each 100-seed lot was placed in a 2 1/4" x 3 1/2" envelope and all the seed lots treated at 176° or 194°F. were put in the oven at the same time and some of them were taken out after each of the four durations. The treated seed lots and four untreated (check) 100-seed lots from each variety were germinated. The high temperature treatment increased the permeability of some hard seed and caused them to germinate. In order to eliminate the effect of this factor on the results, the number of hard seeds in every treated and check seed-lot was counted at the end of the germination period and the number added to the final germination count to give the final germination percentage. Seed-testing laboratories include the number of hard seed in the final germination percentage of alfalfa seed.

The relative germination percentage of the treated seed was considered an index of the hardiness of the variety. The seedlings which germinated did not show any visible abnormality as a result of the treatment.

Intravarietal Selection Studies

Three 100-seed lots from each of the seven varieties were germinated in a seed germinator under a moisture tension of 12 atmospheres produced by mannitol. The first 14 seeds which germinated from each variety were washed thoroughly in tap water and then transplanted in 7 pots. The pots were partially filled with soil which was then saturated with Hyponex solution, two of the selected seeds were transplanted in each pot, and were covered with a 1/2" layer of sand.

After a germination period of 7 days, the seeds which did not germinate under 12 atmospheres tension were washed thoroughly in tap water and germinated in 0 atmosphere tension (tap water). Germination proceeded very rapidly upon the transfer of the seed from the 12-atmosphere tension to tap water and during one day most of transferred seed germinated, indicating that the viability of the seeds was not affected by remaining for a week in the concentrated mannitol solution. Fourteen of the seedlings were then transplanted in 7 pots in a similar manner to that of the "12" selections.

Later all the pots were thinned to leave only one plant per pot. The selected plants were grown under normal greenhouse conditions. Five "0" plants and five "12" plants for each variety, chosen at random, were used in the studies described in the following sub-sections.

When the selected plants were 4 1/2 months old, 25 cuttings were made from each plant. The cuttings were rooted in sand flats and after 3 weeks, 10 cuttings for each plant were transplanted in 1 1/2" x 1 1/2" x 2 3/8" soil bands. Three weeks later, the soil bands were transplanted in a modified split plot design in a Sarpy fine sandy loam soil at the Ashland Farm, Manhattan, Kansas, in the first week of May, 1961. However, due to unawareness

in the previous use of the land, the residual effect of some chemicals resulted in the loss of a part of the planting and some Teton plants had to be transplanted four weeks later. The proper statistical analysis for the remaining part of the planting was followed in analyzing the data. The plants were grown under supplemental sprinkling irrigation and after about two months from the first date of transplanting, the tops of all the plants were harvested. The harvested tops were stored at $40^{\circ} \pm 2^{\circ}$F. and all the leaves from each plant were removed. The leaves and stems were then dried in the oven and weighed separately. Both the leaf to stem ratio (dry-weight basis) and the total dry weight of each plant were calculated.

The plants in the original five-plant selection groups were intercrossed when they started to flower. However, only five varieties (Buffalo, Ladak, Rhizoma, African, and Nomad) produced a sufficient number of flowers under normal greenhouse conditions in April, May and June. Therefore only these varieties were included in the following experiments.

The mature pods on each selected plant were harvested separately, dried in a drier at 100°F. for a few hours and then threshed between two corrugated-rubber surfaces. The seed was scarified in a sand-paper scarifier using an air pressure of 20 lbs. for 30 seconds. Sufficient amounts of seed were produced from a number of plants ranging between three and five from each intercrossed group. An equal number of seed from each of these plants were mixed to produce the seed lots which represented that group in the experiments described in the following sub-sections.

Three 20-seed lots from each selection group were germinated under 12 atmospheres of moisture stress, produced by mannitol, in a completely randomized design. Single 20-seed lots were germinated in tap water and served as checks. Daily germination counts were made in the same manner described

earlier. The length of radicle and hypocotyl was measured for all the seedlings in the 12-atmosphere Petri dishes and for 6 seedlings taken at random from each 0-atmosphere Petri dish. All the seed lots germinated in tap water had 100 percent germination.

Two 18-seed lots from each selection group were planted in pots in a similar manner to that described under the high-temperature tests except that daily emergence counts were made until most of the seedlings emerged and the final emergence counts were made 13 days after planting. The seedlings were then thinned to 15 seedlings per pot except one of the Ladak "0" pots which had 13 seedlings suitable for testing. The plants were tested at the age of two weeks in the heat room for 12 hours, starting at 10 a.m., in the same manner as before. The temperature, relative humidity, and maximum soil temperatures were 128°- 130°F., 29-31 percent, and 113°F., respectively. The seedlings were allowed to recover for two weeks after which the surviving seedlings were counted.

A similar set of pots was tested in the same manner mentioned above except that the heat test was for 12.5 hours at a temperature of 127°- 128°F. with a relative humidity of 33-38 percent and the maximum soil temperature was 109°F. In this set, the number of seedlings per pot was 15, except for the two Ladak "0" pots in which only eight and 10 seedlings were suitable for testing.

In both sets, the "12" F_1 seedlings emerged earlier than the "0" F_1 seedlings and therefore the two classes of seedlings were not in exactly the same stage of development at the time of the heat test. All the pots were planted and the seedlings grown in the cooled greenhouse, mentioned earlier, during August.

All the data obtained throughout the investigation were analyzed statistically following the procedures outlined by Snedecor (98).

EXPERIMENTAL RESULTS

A better interpretation of the results should be obtained if the relationships among the results from the different experiments are considered. Therefore, the discussion of the results is presented in a later section. The statistical analyses of the data are included in an appendix.

Germination and Seedling Growth in Mannitol

The rate of germination, final germination percentage and seedling growth are shown in Table 2. The data for final germination and seedling growth were expressed as percent of check (0 atmosphere tension) and these were analyzed statistically. These data also are included in Table 2, and statistical analysis is presented in the appendix (Table 17).

The differences in final germination among varieties and among tensions were significant at the 1 percent level of probability. However, the variety x tension interaction also was significant at the 1 percent level indicating that the comparison among the varieties varies significantly under the different moisture tensions. The 5-day germination data also were analyzed statistically in the same manner and the results of the analysis were similar to those for the final germination and therefore they are not presented. Both final and rate of germination were reduced with an increase in moisture tension. The data in Table 2 show that in general the reductions are less pronounced in African and Buffalo, the less winterhardy varieties, than in the other five varieties. However, the highly significant interactions represent a decided disadvantage in the use of the method to test the hardiness (to cold, drought, or even heat) of these varieties, since the varieties compare differently under the different tensions.

Table 2. Rate of germination, final germination and seedling growth for seven alfalfa varieties under four moisture tensions.

Variety	Moisture Tension, Atmospheres	Germination Percentage, Days: 1	2	3	4	5	6	7	Final, Percent of check	Seeding growth: mm.	Percent of check
Teton	0	1	61	84	86	87	88	88	100	45	100
	4	0	13	53	64	77	82	83	95	26	57
	8	0	1	10	17	29	43	49	56	12	28
	12	0	0	0	2	5	15	20	23	10	23
Buffalo	0	6	80	89	90	90	90	90	100	53	100
	4	0	22	62	72	78	83	85	94	35	67
	8	0	1	17	37	50	65	73	81	19	36
	12	0	0	2	8	21	35	45	50	14	26
Ladak	0	1	51	76	79	81	82	82	100	48	100
	4	0	8	35	51	71	79	82	101	29	59
	8	0	0	8	16	31	40	48	59	15	31
	12	0	0	0	2	9	17	25	30	11	23
Rhizoma	0	0	45	81	88	89	89	90	100	46	100
	4	0	12	51	65	76	81	82	92	26	57
	8	0	1	12	21	34	44	49	55	14	30
	12	0	0	1	3	14	25	34	37	10	22
Rambler	0	0	38	70	75	78	79	80	100	49	100
	4	0	5	36	56	69	75	78	98	27	54
	8	0	0	10	16	26	38	47	58	14	28
	12	0	0	0	0	4	16	25	31	11	21

Table 2 (concl.)

Variety	Moisture Tension, Atmospheres	Germination Percentage								Seedling growth	
		Days							Final, Percent of check	mm.	Percent of check
		1	2	3	4	5	6	7			
African	0	8	72	86	87	88	88	88	100	57	100
	4	1	39	75	83	86	87	87	99	34	59
	8	0	12	43	63	76	81	84	95	22	39
	12	0	0	5	12	29	51	61	69	13	23
Nomad	0	3	59	85	89	89	89	89	100	52	100
	4	1	29	59	69	76	82	84	95	25	49
	8	0	4	14	25	39	50	57	64	16	31
	12	0	0	3	14	20	27	32	36	11	22
LSD, .05									12.7		11.5
C.V.									12.0		15.4

The 4-atmosphere tension reduced significantly the 5-day germination but did not greatly reduce final germination, indicating that this moisture stress reduced the rate of germination to a greater extent than final germination. The 8- and 12-atmosphere tension reduced both the 5-day and final germination significantly.

Statistical analysis of the seedling-growth data showed that the differences among the varieties and the variety x tension interaction were not significant, indicating that an increase in moisture tension caused a similar relative reduction in the seedling growth of the different varieties. The significance of these results is discussed later. The differences among tensions were significant at the 1 percent level and all three moisture tensions reduced seedling growth significantly. It is of interest, however, to note that a moisture stress as low as 4 atmospheres caused, on the average, a reduction of 42.6 percent in seedling growth. The reductions in seedling growth due to the 8- and 12-atmosphere were, on the average, 68.3 and 77.2 percent, respectively.

Germination and Seedling Growth in Mannitol and Gibberellic Acid

Results of this experiment are presented in Table 3. Statistical analysis of the data is shown in Table 18.

The three varieties which were chosen for this study represented a wide range of winter hardiness and vigor. African is a non-hardy, rapid-growing variety, Ladak is a very hardy, relatively slow-growing variety, and Buffalo is intermediate between African and Ladak.

As in the previous experiment, differences in final germination among varieties and among tensions, and the variety x tension interaction were

Table 3. Rate of germination, final germination and seedling growth for three alfalfa varieties under four moisture tensions and four gibberellic acid concentrations.

Variety	G.A., ppm.	Moisture Tension, Atmospheres	Germination Percentage — Days 1	2	3	4	5	6	7	Final, Percent of check	Seedling growth mm.	Seedling growth Percent of check
Buffalo	0	0	63	86	88	89	89	89	89	100	59	100
		4	4	53	82	85	85	86	86	97	39	67
		8	0	7	26	57	59	66	74	83	25	43
		12	0	0	0	9	16	22	29	33	12	21
	50	0	46	81	85	85	85	85	85	96	57	97
		4	0	38	82	87	87	87	88	98	37	63
		8	0	3	31	65	69	72	73	81	26	43
		12	0	0	0	12	21	25	32	36	15	26
	100	0	33	80	83	85	86	86	86	96	62	105
		4	1	60	87	89	89	89	189	100	43	73
		8	0	3	36	68	74	77	79	88	26	44
		12	0	0	0	10	23	29	39	44	14	24
	150	0	34	85	88	88	89	89	89	99	63	107
		4	0	36	81	87	87	88	89	99	42	72
		8	0	7	24	60	64	66	68	76	28	47
		12	00	0	0	15	24	28	34	38	17	28

Table 3. (cont.)

Variety	G.A., ppm.	Moisture Tension, Atmospheres	Germination Percentage								Seedling growth	
			Days							Final, Percent of check	mm.	Percent of check
			1	2	3	4	5	6	7			
Ladak	0	0	20	62	74	76	77	78	79	100	54	100
		4	0	26	56	67	68	70	74	93	36	67
		8	0	0	5	25	32	37	45	57	16	29
		12	0	0	0	3	5	6	11	14	10	18
	50	0	14	67	72	76	78	82	84	106	56	104
		4	0	20	60	74	77	78	79	100	30	55
		8	0	1	4	29	40	45	49	61	20	37
		12	0	0	0	4	7	11	17	21	12	21
	100	0	7	62	72	77	78	80	81	102	56	104
		4	0	18	51	74	79	81	82	103	42	77
		8	0	3	7	32	40	46	56	70	29	54
		12	0	0	1	5	7	11	18	22	13	23
	150	0	9	66	73	74	75	75	76	96	55	103
		4	0	38	63	76	79	80	81	102	39	73
		8	0	1	5	31	44	49	53	67	25	46
		12	0	0	0	5	8	9	12	15	14	26

Table 3. (concl.)

Variety	G.A., ppm.	Moisture Tension, Atmospheres	Germination Percentage								Seedling growth	
			Days							Final, Percent of check	mm.	Percent of check
			1	2	3	4	5	6	7			
African	0	0	70	88	90	91	91	91	91	100	60	100
		4	24	78	82	82	83	83	83	92	43	72
		8	0	47	71	78	81	82	83	92	29	48
		12	0	6	14	45	54	60	64	70	14	24
	50	0	58	78	81	83	83	83	84	92	59	98
		4	16	81	84	86	87	87	87	96	38	64
		8	0	44	71	78	81	81	82	90	26	43
		12	0	1	12	48	56	58	66	73	16	27
	100	0	41	80	83	85	85	85	86	95	60	100
		4	13	80	83	84	84	84	85	93	45	75
		18	00	39	51	69	74	76	79	87	31	52
		12	0	4	15	38	43	48	51	57	18	30
	150	0	46	83	86	87	87	87	87	96	64	107
		4	12	81	85	87	87	87	87	96	45	76
		8	0	58	72	82	83	85	86	95	28	47
		12	0	4	14	55	63	64	66	73	19	32
LSD, .05										10.6		8.0
C.V.										8.3		8.2

significant at the 1 percent level. The variety x gibberellic acid interaction also was significant at the 1 percent level indicating that the three varieties responded differently to gibberellic acid. The data in Table 3 show that the relative stimulating effect of gibberellic acid on final germination is highest in Ladak, lowest in African, and intermediate in Buffalo. Gibberellic acid had no effect beyond that of the interaction. The tension x gibberellic acid interaction was not significant indicating that the responses to gibberellic acid were similar under the different moisture tensions. The second-order interaction also was not significant indicating that the first-order interactions did not vary with the different treatments or varieties.

Statistical analysis of the 5-day germination data is not presented because the results were similar to those of final germination, except that the effect of gibberellic acid was significant at the 10 percent level of probability, indicating that gibberellic acid had a greater stimulating effect on the rate of germination than on final germination. The variety x gibberellic acid interaction approached significance at the 5 percent level.

On the other hand, the effect of gibberellic acid on seedling growth was significant at the 1 percent level. The 50 ppm. concentration had no stimulating effect. The higher concentrations (100 and 150 ppm.) had similar significant stimulating effects. It is of interest to note that the variety x gibberellic acid in this case was not significant, indicating that the three varieties responded similarly to gibberellic acid. The difference in the response of the three varieties to gibberellic acid with respect to seedling growth in comparison with that of final germination indicates a possible differential effect of the seed coat on the germination process of these varieties, which will be discussed later.

Statistical analysis of the seedling-growth data showed that the tension x gibberellic acid interaction was significant at the 1 percent level, indicating that the stimulating effect of gibberellic acid was dependent on the moisture tension. The data in Table 3 show that the relative stimulating effect of gibberellic acid increased with an increase in moisture tension. Variety differences were significant at the 1 percent level but these differences represent confounded responses to both moisture tension and gibberellic acid. The variety x tension interaction was not significant and this substantiated a similar result in the previous experiment. The second-order interaction was significant only at the 10 percent level.

Hardening in Mannitol

The survival percentages of seedlings germinated at "0" and "6" atmospheric tension after different periods of testing are reported in Table 4. The statistical analysis of these data is presented in the appendix (Table 19).

Table 4. The survival percentages of hardened and unhardened seedlings of seven alfalfa varieties after five testing durations.

Duration (hours)	Hardening (atmos.)	Teton	Buffalo	Ladak	Rhizoma	Rambler	African	Nomad
4	0	75	90	80	80	95	75	75
	6	80	80	45	65	70	95	70
8	0	60	70	85	90	100	55	50
	6	70	75	70	80	85	80	85
12	0	70	70	85	65	95	80	70
	6	95	65	45	65	80	90	85
16	0	45	80	55	60	60	40	20
	6	70	60	55	75	65	55	70
20	0	15	60	40	50	60	30	45
	6	80	70	55	65	70	90	70

C.V. = 24.8

The main interest in the statistical analysis of the data of this experiment was the variety x hardening interaction which was significant at the 1 percent level. This indicated that the reaction of the seedlings to the hardening treatment varied differentially with the different varieties. The analysis of this interaction is shown in Table 5 in which the survival percentages were averaged over the five testing durations. The "6" seedlings of Teton, African, and Nomad had significantly higher survival percentages than the "0" seedlings, suggesting that the seedlings of these varieties were able to harden. This was not true in the case of the seedlings of Buffalo, Ladak, Rhizoma, and Rambler. Therefore, the relative ranking of the varieties was quite different in the hardened than in the unhardened condition.

Table 5. The mean survival percentages of hardened (6-atmosphere) and unhardened (0-atmosphere) seedlings of seven alfalfa varieties.

Hardening	Varieties						
(atmos.)	Teton	Buffalo	Ladak	Rhizoma	Rambler	African	Nomad
0	53	74	69	69	82	56	52
6	79	70	54	70	74	82	76

LSD, .05, between any two values in the table = 15.2

The difference between the hardening and the unhardening treatments was significant at the 5 percent level and in favor of the hardening treatment, indicating that the over-all effect of the hardening treatment was to increase the hardiness of the seedlings. The differences among the varieties beyond those of the interaction were significant only at the 10 percent level. The differences among the testing durations were significant at the 1 percent level. The 4-, 8-, and 12-hour testing periods were not significantly

different from each other, caused less kill, and each one of them was significantly different from the 16- and 20-hour periods which caused more kill and were not significantly different from each other. The variety x duration and the hardening x duration interactions were not significant, indicating that the comparisons among the varieties and the comparison between the hardening treatments were similar under the different testing periods. The second-order interaction was not significant, indicating that the first-order interactions were independent of the treatments or varieties.

Growth in Mannitol-Nutrient Solution

All the data of this experiment were expressed as percent of check (0 atmospheres tension) to exclude the effects of the original differences among the varieties from the varietal comparisons. The data for dry-matter yield, the contents of nitrogen, phosphorus, and potassium, and the yields of nitrogen, phosphorus, and potassium are presented in Tables 6, 7, and 8 respectively. Statistical analyses are included in the appendix (Tables 20, 21, and 22 respectively).

In dry-matter yield, differences among the varieties and the variety x tension interaction were not significant. The differences among tensions and among tanks were significant at the 1 percent level. Upon the addition of mannitol to the nutrient solution, the plants wilted and the degree of wilting was proportional to the moisture stress inflicted. However, the plants in the 4-atmosphere tension made apparent recovery from wilting. After few days firing of tops of plants under the 8- and 12-atmosphere tensions occurred. This was particularly severe in the 12-atmosphere tanks. Therefore, the time which was available for the physiologic changes associated

with drought to occur increased with the decrease in moisture tension from 12 to 4 atmospheres. This has a bearing on the observed decrease in relative dry-matter yields of tops with the decrease in moisture stress from 12 to 4 atmospheres which is believed to be due to breakdown of carbohydrates in the leaves (52) accompanied by their deposition as food reserves in the roots under conditions of limited moisture supply. Large accumulations of carbohydrates in roots under drought conditions have been reported in cotton (30) and in several grass species (55).

Table 6. Dry-matter yields of tops of plants of seven alfalfa varieties grown in nutrient solution under four moisture tensions, expressed as percent of check.

Variety	Moisture Tension, atmospheres				Variety mean
	0	4	8	12	
Teton	100	70	95	96	90
Buffalo	100	80	94	90	91
Ladak	100	79	73	82	83
Rhizoma	100	65	79	76	80
Rambler	100	81	72	83	84
African	100	84	78	93	89
Nomad	100	72	85	87	86
Tension mean	100	76	82	87	

LSD, .05, for tension means = 7.3
The differences among variety means were N.S.
C.V. = 13.7

Differences in nitrogen content (percentage) among the varieties were not significant but those of the nitrogen yield (°/o N x dry matter) were significant

at the 5 percent level. The order of the varieties with respect to their mean relative nitrogen yield (highest to lowest) was: African, Teton, Nomad, Rambler, Buffalo, Rhizoma, and Ladak. The differences in both nitrogen content and nitrogen yield among the tensions were significant at the 1 percent level. There was a progressive decrease in nitrogen uptake with an increase in moisture stress.

The differences among the varieties in phosphorus content were significant at the 5 percent level and those in phosphorus yield were significant at the 1 percent level. The varieties ranked in the same order, in respect to their relative content and yield of phosphorus, as follows (highest to lowest): Nomad, Rhizoma, Buffalo, African, Teton, Rambler, and Ladak. The differences between the tensions in both phosphorus content and yield were significant at the 1 percent level. There was a progressive decrease in phosphorus content with the increase in moisture tension. The only exception to the general trend of decreasing nutrient content and yield, and therefore nutrient uptake, with the increase in moisture tension in this experiment was the yield of phosphorus under the 4-atmosphere tension which was slightly less than that under the 8-or 12-atmosphere tension but the differences were not significant.

Differences among varieties in both content and yield of potassium were significant at the 1 percent level. The importance of potassium content in drought resistance of plants (67) is greater than the importance of potassium yield in the nutritional value of the crop. With respect to potassium content, the varieties ranked in the following order (highest to lowest): Nomad, Rhizoma Teton, Buffalo, African, Rambler, and Ladak. The differences among the tensions in both the content and yield of potassium were significant at the 1 percent level. An increase in moisture stress caused a progressive decrease in potassium uptake.

Table 7. Contents (percentages) of nitrogen, phosphorus, and potassium in tops of plants of seven alfalfa varieties grown in nutrient solution under four moisture tensions, expressed as percent of check.

Nutrient	Variety	Moisture tension, atmospheres 0	4	8	12	Variety mean
Nitrogen	Teton	100	96	66	66	82
	Buffalo	100	76	64	66	76
	Ladak	100	73	76	63	78
	Rhizoma	100	87	78	76	85
	Rambler	100	85	80	70	84
	African	100	79	93	74	87
	Nomad	100	95	78	64	84
	Tension mean	100	84	76	68	
LSD, .05, for tension means = 7.7 Differences among variety means were N.S. C.V. = 8.4						
Phosphorus	Teton	100	86	61	63	77
	Buffalo	100	71	73	73	79
	Ladak	100	66	73	64	76
	Rhizoma	100	91	86	86	91
	Rambler	100	71	68	68	77
	African	100	67	82	67	79
	Nomad	100	106	84	78	92
	Tension mean	100	80	75	71	
LSD, .05, for variety means = 12.1 LSD, .05, for tension means = 9.2 C.V. = 10.0						
Potassium	Teton	100	73	49	49	68
	Buffalo	100	61	51	44	64
	Ladak	100	55	60	45	65
	Rhizoma	100	87	80	73	85
	Rambler	100	61	50	48	65
	African	100	52	60	43	64
	Nomad	100	97	67	61	81
	Tension mean	100	69	59	52	
LSD, .05, for variety means = 12.6 LSD, .05, for tension means = 9.5 C.V. = 12.0						

Table 8. Yields (% N x dry matter) of nitrogen, phosphorus, and potassium of tops of plants of seven alfalfa varieties grown in nutrient solution under four moisture tensions, expressed as percent of check.

Nutrient	Variety	Moisture Tension, atmospheres 0	4	8	12	Variety mean
Nitrogen	Teton	100	66	63	63	73
	Buffalo	100	61	60	59	70
	Ladak	100	57	55	51	66
	Rhizoma	100	57	61	58	69
	Rambler	100	69	57	58	71
	African	100	67	73	68	77
	Nomad	100	68	66	55	73
	Tension mean	100	64	62	59	
LSD, .05, for variety means = 5.6						
LSD, .05 for tension means = 4.3						
C.V. = 5.3						
Phosphorus	Teton	100	60	58	60	69
	Buffalo	100	57	68	66	73
	Ladak	100	52	53	52	64
	Rhizoma	100	59	67	65	73
	Rambler	100	57	49	57	66
	African	100	57	64	62	71
	Nomad	100	76	72	68	79
	Tension mean	100	60	62	61	
LSD, .05, for variety means = 7.04						
LSD, .05, for tension means = 5.3						
C.V. = 6.7						
Potassium	Teton	100	51	46	47	61
	Buffalo	100	49	48	40	59
	Ladak	100	43	44	37	56
	Rhizoma	100	56	63	56	69
	Rambler	100	50	36	40	56
	African	100	44	47	40	58
	Nomad	100	70	57	53	70
	Tension mean	100	52	49	45	
LSD, .05, for variety means = 7.7						
LSD, .05, for tension means = 5.8						
C.V. = 8.5						

Due to the interferences mentioned earlier (uptake of mannitol and growth of fungi), the results of this experiment are not considered conclusive.

Drought Tests in Flats

Preliminary tests in soil flats for the use of this method to determine the relative drought resistance of the seven varieties indicated differential drying of the soil in the same flat. Therefore, sand and vermiculite were used in the tests mentioned earlier because of their greater uniformity. The data obtained from these tests are not presented because of the high degree of variability (C.V.= 32.5 to 41.0). The method proved to be of little or no value for two main reasons. First, under greenhouse conditions, the outer rows dried faster than the rows in the middle of the flat in spite of the frequent rotating of the flats and even changing their position in the last test. The second reason, is that the varieties differed in their vigor and therefore the more vigorous varieties shaded the less vigorous varieties and suppressed their growth, especially when the plants were in an advanced stage of growth.

Emergence and Growth Under Different Temperatures

This and the following two experiments were conducted to obtain information on the reaction of the seven varieties under high temperatures to supplement the data on their reactions to drought. The data for rate of emergence during the first week after the beginning of emergence and for final emergence, 15 days from planting, are presented in Table 9 for the three temperatures. Results for final emergence, dry-weight and nitrogen

content of tops under the 70°- 70° and 90°- 70°F. temperatures were expressed as percent of check (70°- 70°F.) for statistical analysis in order to exclude the original varietal differences from the comparisons among the varieties. These data are shown in Table 10 and statistical analysis is presented in the appendix (Table 23). Data of the 110°- 70°F. temperature were not analyzed because of obvious differences.

Table 9. The rate of emergence and final emergence of seven alfalfa varieties under three temperatures*, expressed as the number of seedlings emerged from 60 seeds.

Variety	Temp. (°F.)	Days after planting 2	3	4	5	6	7	8	15
Teton	70-70	6	20	28	34	44	49	51	52
	90-70	0	2	2	10	24	36	38	39
	110-70	0	0	0	0	0	0	1	1
Buffalo	70-70	12	30	37	42	47	48	48	48
	90-70	1	3	6	16	32	39	42	43
	110-70	0	0	0	0	1	5	8	14
Ladak	70-70	0	4	8	19	28	36	40	48
	90-70	0	7	14	17	25	34	39	46
	110-70	0	0	0	0	0	0	1	7
Rhizoma	70-70	0	6	9	18	32	43	45	48
	90-70	0	3	5	8	21	34	39	44
	110-70	0	0	0	0	0	0	0	1
Rambler	70-70	1	13	18	26	33	37	38	40
	90-70	0	8	16	19	25	31	34	36
	110-70	0	0	1	1	2	3	4	4
African	70-70	16	32	36	42	48	51	51	51
	90-70	3	16	18	24	27	38	39	41
	110-70	0	0	0	0	1	5	7	8
Nomad	70-70	2	12	19	32	46	50	51	51
	90-70	1	5	6	10	21	29	32	34
	110-70	0	0	0	0	1	2	3	5

*First figure is day (16 hr.) temperature and second is night (8 hr.) temperature

The difference in final emergence between the 70°- 70° and 90°- 70°F. temperatures was significant at the 1 percent level. A similar statistical analysis was made on the data of emergence on the seventh day after planting and the analysis is not presented because the results were similar to those of final emergence. The results indicated that both the final and rate of emergence were reduced significantly by the 90°- 70°F. temperature. The differences among the varieties and the variety x temperature interaction were not significant in either analysis. The data in Table 9 show that the final and rate of emergence were drastically reduced by the 110°- 70°F. temperature.

For differences in dry weight of tops, variety, temperature, and the variety x temperature interaction were not significant. Periodic height measurements, however, showed that the plants under the 90°- 70°F. temperature were growing faster in length, but they were more spindly, than the plants under the 70°- 70°F. temperature. The plants under the 110°- 70°F. made very little growth and many of them died before the end of the experiment.

Table 10. Final emergence, dry weight of tops and nitrogen content of seven alfalfa varieties under two temperatures*, expressed as percent of check.

Variety	Final emergence: Temperature (°F.) 70-70	90-70	Variety mean	Dry weight of tops: Temperature (°F.) 70-70	90-70	Variety mean	Nitrogen content: Temperature (°F.) 70-70	90-70	Variety mean
Teton	100	74	87	100	90	95	100	99	99
Buffalo	100	89	95	100	102	101	100	90	95
Ladak	100	96	98	100	122	111	100	89	95
Rhizoma	100	91	96	100	112	106	100	88	94
Rambler	100	89	95	100	110	105	100	92	96
African	100	80	90	100	97	99	100	99	99
Nomad	100	66	83	100	94	97	100	91	95
Temp.mean	100	84		100	104		100	92	
LSD, .05	5.9		N.S.	N.S.		N.S.	2.8		N.S.
C.V.	10.2			13.9			4.6		

*First figure is day (16 hr.) temperature and second is night (8 hr.) temperature.

The difference in nitrogen content between the 70°- 70° and 90°- 70°F. were significant at the 1 percent level in favor of the 70°- 70°F. temperature. Differences among the varieties, and the variety x temperature interaction were not significant.

High-Temperature Tests

The results of these tests showed considerable variation. The data from the tests are not presented because of the high coefficients of variation (C.V.= 27.3 to 107.9) and the lack of correlation between the ranking of the seven varieties in the different tests. The data, however, indicated that precise control of temperature and humidity during the tests would be necessary if consistent results are to be obtained.

Dry-Heat Treatment of Seed

The germination data from this experiment were expressed as percent of check and these were used for the statistical analysis. The data are reported in Table 11 and statistical analysis is included in the appendix (Table 24).

The differences among the varieties, temperatures, and durations were significant at the 1 percent level. The 194°F. temperature caused a significant reduction in germination compared with the 176°F. temperature. The 176°F. treatment caused only a slight reduction in germination, and therefore a better separation of the varieties was obtained with the 194°F. than with the 176°F. temperature. The 60-minute duration reduced germination significantly as compared with the 30-minute duration. The treatments for 90 and 120 minutes reduced germination significantly as compared with either the 30- or 60-minute treatments. The difference between the effects of the 90- and 120-minute durations was not significant but the reduction in germination was

less with the 120- than with the 90-minute treatment, indicating a possible hardening to the treatment after an initial exposure to it, but no positive conclusion can be made.

Table 11. Germination of seed of seven alfalfa varieties after dry-heat treatment of seed at two temperatures and four durations, expressed as percent of check.

	Temperature, °F.								
	176				194				
	Duration, minutes								Variety
Variety	30	60	90	120	30	60	90	120	mean
Teton	105	102	97	100	102	99	101	94	100
Buffalo	94	100	100	96	96	83	78	75	90
Ladak	98	101	100	95	89	78	61	53	84
Rhizoma	103	99	98	96	84	53	35	58	78
Rambler	101	97	95	95	89	84	59	80	87
African	97	96	94	98	90	78	81	86	90
Nomad	101	96	101	100	77	76	70	74	87
LSD, .05									4.7
C.V.= 9.3									

The variety x temperature interaction was significant at the 1 percent level and the variety x duration interaction was significant at the 5 percent level, indicating that the comparison among the varieties depends on the temperature or duration used, respectively. The temperature x duration interaction was significant at the 1 percent level, indicating that the effect of duration varies with the different temperatures. The reduction in germination increased progressively with the increase in time of duration with the 176°F. treatment, but the effects of the four durations were not significantly different. With the 194°F. temperature, the 60-, 90- and 120-minute treatments caused significant reductions in germination as compared with 30-minute duration. The treatment for 90 minutes reduced germination significantly as compared

with the 60-minute duration while the 120-minute treatment caused a non-significant reduction as compared with the treatment for 60 minutes. The 90-minute duration caused a greater reduction in germination than the treatment for 120 minutes but the difference was not quite significant at the 5 percent level. The variety x temperature x duration interaction was almost significant at the 5 percent level, indicating that the first order interactions depend on the treatment or variety involved.

Intravarietal Selection Studies

The main interest in the results of these studies was the difference between the 0-atmosphere and 12-atmosphere selections and the variety x selection interaction. Data for the leaf to stem ratio and dry-matter yield of the "0" and "12" selections are presented in Table 12. The statistical analyses for the leaf to stem ratios and for mean dry-matter yields are included in the appendix (Tables 25 and 26, respectively). The means of dry-matter yield for the replications were analyzed statistically instead of the dry-matter yields of the individual plants because the latter varied considerably.

In the statistical analysis of the leaf to stem ratios the variety x selection interaction was significant at the 1 percent level, indicating a differential effect of selection of the leaf to stem ratio of the different varieties. The ratio was significantly less in the "12" selections than in "0" selections of Teton and Ladak. Rhizoma showed a similar trend to that of Teton and Ladak, but the difference was not significant between the two selections. On the other hand, the ratio was higher in the "12" selections than in the "0" selections of Rambler, African, Nomad, and Buffalo but the differences were not significant. The differences among the varieties beyond

those of the interaction were not significant. The difference between the selections beyond that of the interaction was significant only at the 10 percent level. The differences between plants within the varieties and selections were significant at the 1 percent level.

Statistical analysis of the mean dry-matter yields showed that the variety x selection interaction was significant only at the 10 percent level. The differences among the varieties and between the selections, beyond those of the interaction, were not significant.

Table 12. Leaf to stem ratios and dry-matter yields of two selections of seven alfalfa varieties.

Variety	Leaf to stem ratio: Selection "0"	Leaf to stem ratio: Selection "12"	Dry-matter yield, gms. per plant: Selection "0"	Dry-matter yield, gms. per plant: Selection "12"
Teton	2.268	1.872	3.33	6.43
Ladak	1.660	1.355	10.46	7.71
Rambler	1.361	1.363	10.72	11.90
African	1.111	1.255	11.96	8.72
Nomad	1.386	1.461	10.85	8.80
LSD, .05	0.1509		N.S.	
Buffalo	1.544	1.640	12.30	11.26
Rhizoma	1.468	1.418	7.99	9.18
LSD, .05	0.1232		N.S.	

C.V. leaf:stem ratio, 16.1
dry-matter yield, 15.7

The data for the rate of germination, final germination and seedling growth of the seed from the "0" and "12" selections under 12 atmospheres of moisture stress are reported in Table 13. The data for seedling growth were expressed as percent of check and these were analyzed statistically. Since

all the seed had 100 percent germination in tap water, the actual 5-day and final germination percentages were used for the respective statistical analyses. The statistical analyses are included in the appendix (Table 27).

Table 13. The rate of germination, final germination percentage and seedling growth, under a moisture tension of 12 atmospheres, of the seed from two selections of five alfalfa varieties.

Variety	Selection	Germination percentage				Seedling growth	
		Days					Percent
		4	5	6	7	mm.	of check
Buffalo	"12"	1.7	8.3	13.3	16.7	13	18
	"0"	0	3.3	3.3	8.3	6	9
Ladak	"12"	3.3	11.7	11.7	13.3	10	14
	"0"	0	0	0	0	0	0
Rhizoma	"12"	10.0	15.0	25.0	35.0	13	21
	"0"	8.3	11.7	21.7	21.7	14	22
African	"12"	35.0	51.7	63.3	70.0	16	18
	"0"	8.3	8.3	10.0	18.3	14	18
Nomad	"12"	1.7	6.7	10.0	11.7	13	19
	"0"	0	0	0	1.7	3	4
LSD, .05			10.54		13.28		N.S.
C.V.			52.9		39.6		38.3

The results obtained from statistical analyses of the final and 5-day germination data were similar. Differences between the "0" and "12" selections were significant at the 1 percent level, indicating that the ability to germinate under the 12-atmosphere tension was inherited. The differences among the varieties were significant at 1 percent level. The variety x selection interaction also was significant at the 1 percent level indicating a differential degree of heritability in the different varieties. The rate of germination

of Ladak and African and the final germination of Ladak, African and Rhizoma were increased significantly upon selection. The increases in the other cases were not significant.

Differences in seedling growth of the F_1 seedlings of the two selections were significant at the 1 percent level in favor of the "12" F_1 seedlings, indicating that these seedlings had greater vigor. Differences among the varieties were significant at the 1 percent level and the variety x selection interaction was significant at the 10 percent level.

Emergence rate and final emergence of F_1 seedlings of the two selections are shown in Table 14. Statistical analyses for the 3-day and final emergence are presented in the appendix (Table 28). The main interest in these data was the rate of emergence, which was considered as another measure of seedling vigor.

Table 14. The rate of emergence and final emergence of the F_1 seedlings of two selections of five alfalfa varieties, expressed as the number of seedlings emerged from 18 seeds.

Variety	Selection	Days after planting			
		3	4	5	13
Buffalo	"12"	10	17	17	18
	"0"	2	12	16	18
Ladak	"12"	9	16	17	18
	"0"	0	4	9	17
Rhizoma	"12"	10	18	18	18
	"0"	6	13	17	18
African	"12"	16	18	18	18
	"0"	15	18	18	18
Nomad	"12"	5	14	17	18
	"0"	4	13	16	18
LSD, .05		4.6			N.S.
C.V.		41.6			3.2

The difference in the 3-day emergence between the F_1 seedlings of the two selections was significant at the 1 percent level in favor of the "12" F_1 seedlings indicating again that these seedlings had greater vigor. Variety differences were significant at the 1 percent level. The variety x selection interaction was significant at the 5 percent level indicating differential varietal increase in vigor upon selection for ability to germinate under 12 atmospheres of moisture stress produced by mannitol. The data in Table 14 show that significant increases were obtained in Buffalo and Ladak but those of Rhizoma, African and Nomad were not significant.

The differences in final emergence among the varieties and between the selections were not significant and the variety x selection interaction was significant only at the 10 percent level.

Again, due to high degree of variability and lack of correlation between the two high-temperature tests of the F_1 seedlings, the data for these tests are not presented.

DISCUSSION

The growth characteristics and hardiness of the seven alfalfa varieties which were used in this study as reported by Hanson et al. (41) are given in Table 15.

Table 15. Reported growth characteristics and hardiness of the seven alfalfa varieties.

Variety	Characteristics
Teton	Has low, wide crown with aggressive rhizome development. Tends to be moderately dormant after cutting. Becomes dormant early in fall. Very winter hardy.
Buffalo	Upright and quick to recover after cutting. Moderately winter hardy.

Table 15 (concl.)

Variety	Characteristics
Ladak	Semiprocumbent habit of growth. Becomes dormant during prolonged periods of summer drought and in early fall. Recovers slowly after cutting. Very winter hardy.
Rhizoma	Has more or less extensive underground development of crown branches. Very winter hardy.
Rambler	Creeping-rooted and has low-set crown. Drought resistant. Very winter hardy.
African	Extremely non-dormant. Grows late in the fall and early in the spring. Recovers quickly after cutting. Upright in habit of growth. Non-winter hardy.
Nomad	Some plants have well-developed rhizomes, spreading under some conditions. Winter hardy.

The few reports on the relative drought resistance of alfalfa varieties under field conditions are confusing. Pedersen and McAllister (76) reported the relative drought resistance of some varieties under dry farming in 17 locations in Utah. In eight of these locations there was little difference between the varieties tested. Ladak, Ranger, Buffalo or Nomad was the most resistant variety in the other locations. They stated that low-growing types such as Nomad may be able to survive better under dry range conditions. However, they attributed the claimed drought resistance of Nomad to its resistance to gopher root cutting which is due to its ability to regenerate lateral roots. Ladak was found superior where regrowth was limited by drought. This is probably due to its slow recovery after cutting. Pedersen and McAllister (76) concluded that none of the varieties which they tested showed superior resistance to drought. On the other hand, wide differences in the ability of different alfalfa varieties to survive under extremely adverse conditions were brought

out by a hot, dry summer followed by a cold winter and spring drought during the year ending June 1956 in western Canada (24). Severe killing of most tap-rooted alfalfa varieties occurred at Lacombe, Alberta, where drought resistance was the main factor in alfalfa survival in that year. However, since winter crown rot was the final killing agent under those conditions, the better ability of the creeping-rooted alfalfa to survive was attributed to a possible inborne resistance to winter crown rot.

It is evident, then, that the observed relative drought resistance of any alfalfa variety may vary in the different locations because of other factors which are not related to drought.

The basis for using the ability of seed to germinate under moisture tension to test and select for drought resistance originated from the concept that this ability is correlated with a higher osmotic pressure in the plants and therefore greater resistance to drought (35). However, extensive studies with many crop plants, such as that of Newton and Martin (70), showed that osmotic pressure is not a good criterion of drought resistance.

With alfalfa, an opposite relation to that mentioned above was found, the less hardy varieties showed better ability to germinate under moisture stress than the more hardy varieties. Two conceivable explanations for this exist. The first is a greater ability of the more hardy varieties to slow down their growth and become dormant when the moisture supply is limited. This possible explanation is disproved by germination and seedling growth in mannitol. The differences among the varieties in germination were highly significant while the differences in seedling growth were not significant. The other possible explanation is a differential mechanical restriction of the seed-coat in the different varieties and among the seed of the same variety.

This explanation is substantiated by the results of germination and seedling growth in mannitol and gibberellic acid. Gibberellic acid had no significant stimulating effect on germination while its stimulating effect on seedling growth was highly significant. This differential effect of the seed-coat may be due to a differential thickness of the seed-coat and/or to a differential vigor of the germinating embryo. The possibility exists that the more hardy varieties have thicker seed coats than the less hardy varieties but there did not seem to be any reference in the literature to such a relation. That vigor is an important factor in the ability of the seed to germinate under high moisture tension is substantiated by the results of the intra-varietal selection studies. The differences between the F_1 seedlings of the "0" and "12" selections in seedling growth and in the rate of emergence were highly significant in favor of the "12" F_1 seedlings. The relation is also in agreement with the fact that the less hardy varieties are more vigorous than the more hardy varieties.

Although seed-coat characteristics may be another factor besides vigor in the ability to germinate under moisture tension as indicated above, data on leaf-stem ratio indicated that the difference in this ability between a hardy and a non-hardy variety is not due solely to seed-coat characteristics as inferred by Rumbaugh and Swanson (83).

That the reduction in germination under moisture stress is not a measure of drought resistance in the alfalfa varieties studied is further substantiated by the lack of correlation between the rank of the varieties in respect to reduction in germination under moisture tension and their rank in respect to seedling survival in either the hardened or the unhardened condition. The results indicated that the method is of possible use in selection for vigor in some of these varieties.

Although the differences among varieties in dry-matter yield under moisture tension (mannitol in tanks) were not significant, the rank of the varieties with respect to yield under 12 atmospheres of tension had a significant rank correlation coefficient of 0.821 with their rank in respect to their seedling survival in the hardened condition.

It is of interest to note that the rank of the varieties in respect to germination after dry-heat treatment of seed at 194°F. showed significant rank correlation coefficient of 0.786 with their rank in respect to seedling survival in the hardened condition and a highly significant rank correlation coefficient of 0.964 with their rank in respect to dry-matter yield under 12 atmospheres of moisture stress.

The three rank correlation coefficients mentioned above indicated a correlation in the relative hardiness of the varieties in the embryo, seedling, and young-plant stages. Furthermore, the results indicated that the separation between these varieties can be attained only by severe tests, such as 16 atmospheres of moisture stress or 194°F.

The rank of the varieties in the hardened condition, 12 atmospheres of moisture tension, or 194°F. treatment did not correlate with their rank in the unhardened condition. The rank of the varieties in the hardened condition is a better and more realistic measure of their relative hardiness than their rank in the unhardened condition. Therefore, the ranks of the varieties in the hardened condition, 12 atmospheres of moisture stress, and 194°F. treatment are reported in Table 16. The Friedman test (92), which is a two-way analysis of variance by ranks, was used to test the differences between the means of the ranks of the different varieties in these three tests. The test gave a χ_r^2 value of 16.29 which approaches significance at the 1 percent

level, indicating significant differences among varieties. The varieties are arranged in Table 16 in a decreasing order of hardiness as indicated by their respective means of ranks.

Table 16. Relative ranks of seven alfalfa varieties according to their relative hardiness as tested by three different tests.

Variety	Hardening1/	12 Atmospheres2/	194°F.3/	Mean of ranks for variety
Teton	2	1	1	1.3
African	1	2	2	1.7
Buffalo	5	3	3	3.7
Nomad	3	4	5	4.0
Rambler	4	5	4	4.3
Ladak	7	6	6	6.3
Rhizoma	6	7	7	6.7

1/Germination under a moisture tension of 6 atmospheres.
2/Growth under moisture stress.
3/Dry heat treatment of seed.

CONCLUSIONS

1. The ability of the seven alfalfa varieties to germinate under moisture tension produced by mannitol is more related to vigor than to hardiness.

2. This ability is heritable and not due solely to seed-coat characteristics.

3. The tremendous ability of African seed to germinate under high osmotic stresses and the vigor of its seedlings suggests that better stands might be obtained with this variety than with other varieties in the saline soils of the semi-arid regions such as those of Iraq, where winterhardiness is not a factor.

4. A highly significant gibberellic acid x variety interaction indicated that gibberellic acid had a higher relative stimulating effect on the germination of the slower- than of the faster-growing variety.

5. A highly significant gibberellic acid x tension interaction indicated that gibberellic acid had a higher relative stimulating effect on seedling growth under higher than with lower moisture stresses.

6. Teton, African, and Nomad exhibited an ability to harden in the seedling stage to a moisture tension of 6 atmospheres produced by mannitol. Buffalo, Ladak, Rhizoma, and Rambler did not show such an ability.

7. It appeared that uptake of nitrogen, phosphorus and potassium from nutrient solution decreased progressively with the increase in moisture stress.

8. The order of the seven varieties in respect to their ability to take up these nutrients under moisture tension was (highest to lowest): Nomad, Rhizoma, Teton, African, Buffalo, Rambler, and Ladak.

9. The method of testing the drought resistance of these varieties at different stages of growth in flats of soil, sand or vermiculite proved to be unsatisfactory.

10. Day-temperatures of 90° and 110°F. had adverse effects on emergence of alfalfa as compared with 70°F. day-temperature, suggesting that better stands might be obtained with spring than with summer seedings.

11. Although the 90°F. day-temperature had no significant effect on the dry-weight of tops as compared with 70°F., 90°F. significantly reduced the nitrogen content of the tops when compared with that under the 70°F. temperature.

12. Testing the resistance to high temperatures in the seven varieties at different stages of growth in soil pots indicated that an accurate control of temperature and relative humidity must be secured in the heat room or chamber if consistent results are to be obtained.

13. Dry-heat treatment of seed of these seven varieties at 194°F. for 30-120 minutes gave results which correlated with the ability of their hardened seedlings to survive injury by plasmolysis, in a solution with 16 atmospheres of osmotic pressure, and rapid deplasmolysis, in tap water, and with the ability of their young plants to grow under a moisture tension of 12 atmospheres.

14. The relative hardiness of the seven varieties, as measured by the means of their relative ranks in the three tests mentioned above indicated that these varieties fall in three groups: a more hardy group including Teton and African; an intermediate group including Buffalo, Nomad, and Rambler; and a less hardy group including Ladak and Rhizoma.

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APPENDIX

Table 17. Analysis of variance of final germination and seedling growth of seven alfalfa varieties at four moisture tensions.

Source of Variation	d.f.	Mean Square: Final germination	Mean Square: Seedling growth
Varieties	6	980.66**	102.02
Tensions	3	22325.70**	33580.79**
Variety x Tension	18	322.33**	35.40
Error	84	82.13	67.04
Total	111		

**Significant at the 1 percent level.

Table 18. Analysis of variance of final germination and seedling growth of three alfalfa varieties at four moisture tensions and four levels of gibberellic acid.

Source of Variation	d.f.	Mean Square: Final germination	Mean Square: Seedling growth
Varieties	2	3341.21**	143.71**
Tensions	3	25462.84**	39806.69**
Variety x Tension	6	2242.87**	40.31
Gibberellic Acid	3	40.48	543.44**
Variety x Gibberellic Acid	6	140.19**	43.98
Tension x Gibberellic Acid	9	36.28	71.28**
Variety x Tension x Gibberellic Acid	18	45.07	38.43#
Error	96	42.72	24.23
Total	143		

**Significant at the 1 percent level.
#Significant at the 10 percent level.

Table 19. Analysis of variance of survival percentage of hardened and unhardened seedlings of seven alfalfa varieties after five testing durations.

Source of Variation	d.f.	Mean square
Varieties	6	597.38#
Hardening	1	1785.72*
Variety x Hardening	6	1564.05**
Durations	4	2869.65**
Variety x Duration	24	262.56
Hardening x Duration	4	146.96
Variety x Hardening x Duration	24	166.73
Error	70	290.00
Total	139	

**Significant at the 1 percent level.
*Significant at the 5 percent level.
#Significant at the 10 percent level.

Table 20. Analysis of variance of dry-matter yield of seven alfalfa varieties grown under four moisture tensions.

Source of variation	d.f.	Mean square
Varieties	6	191.91
Tensions	3	2193.80**
Variety x Tension	18	121.42
Tanks : Tensions	8	1572.25**
Error	48	139.68
Total	83	

**Significant at the 1 percent level.

Table 21. Analysis of variance of nitrogen, phosphorus, and potassium contents of seven alfalfa varieties grown under four moisture tensions.

Source of Variation	d.f.	Mean Square: Nitrogen	Mean Square: Phosphorus	Mean Square: Potassium
Varieties	6	57.01	190.43*	321.19**
Tensions	3	1287.56**	1144.44**	3137.84**
Error	18	47.20	66.57	71.48
Total	27			

**Significant at the 1 percent level.
*Significant at the 5 percent level.

Table 22. Analysis of variance of nitrogen, phosphorus, and potassium yields of seven alfalfa varieties grown under four moisture tensions.

Source of Variation	d.f.	Mean Square: Nitrogen	Mean Square: Phosphorus	Mean Square: Potassium
Varieties	6	49.60*	98.11**	133.54**
Tensions	3	2603.50**	2679.20**	4741.93**
Error	18	14.39	22.46	26.79
Total	27			

**Significant at the 1 percent level.
*Significant at the 5 percent level.

Table 23. Analysis of variance of final emergence, dry-weight of tops and nitrogen content of seven alfalfa varieties grown at two temperatures.

Source of Variation	d.f.	Mean Square: Final emergence	Mean Square: Dry weight	Mean Square: Nitrogen content
Varieties	6	173.11	192.20	31.09
Temperatures	1	2858.63**	162.05	607.24**
Variety x Tem.	6	173.84	192.24	30.98
Error	28	87.96	200.75	19.73
Total	41			

**Significant at the 1 percent level.

Table 24. Analysis of variance of germination of seed of seven alfalfa varieties treated at two temperatures and four durations.

Source of variation	d.f.	Mean square
Varieties	6	1051.39**
Temperatures	1	17393.14**
Variety x Temperature	6	967.60**
Durations	3	985.51**
Variety x Duration	18	131.05*
Temperature x Duration	3	623.28**
Variety x Temperature x Duration	18	113.96#
Error	112	67.55
Total	167	

**Significant at the 1 percent level.
*Significant at the 5 percent level.
#Significant at the 10 percent level.

Table 25. Analysis of variance of leaf to stem ratio of two selections (0 and 12 atmospheres) of seven alfalfa varieties.

Source of Variation	d.f.	Mean square
Varieties	6	3.1127
Error (a)	9	1.4337
Selections	1	0.2131#
Variety x Selection	6	0.4550**
Plants: Variety and Selection	55	0.3555**
Error (b)	201	0.0592
Total	278	

**Significant at the 1 percent level.
#Significant at the 10 percent level.

Table 26. Analysis of variance of mean dry-matter yield of two selections (0 and 12 atmospheres) of seven alfalfa varieties.

Source of variation	d.f.	Mean square
Varieties	6	23.1289
Error (a)	9	18.2984
Selections	1	1.5295
Variety x Selection	6	5.9054#
Error (b)	9	2.2340
Total	31	

#Significant at the 10 percent level.

Table 27. Analysis of variance of 5-day germination, final germination and seedling growth of seed from two selections of five alfalfa varieties at a moisture tension of 12 atmospheres.

Source of variation	d.f.	Mean square		
		5-day germination	Final germination	Seedling growth
Varieties	4	714.59**	1597.09**	195.66**
Selections	1	1470.00**	2803.34**	420.37**
Variety x Selection	4	417.92**	497.08**	80.53#
Error	20	38.33	60.83	29.61
Total	29			

**Significant at the 1 percent level.
#Significant at the 10 percent level.

Table 28. Analysis of variance of 3-day and final emergence of F_1 seedlings of two selections of five alfalfa varieties.

Source of variation	d.f.	Mean square	
		3-day emergence	Final emergence
Varieties	4	162.96**	0.2875
Selections	1	211.60**	0.9000
Variety x Selection	4	29.29*	0.7125#
Error	30	10.28	0.3167
Total	39		

**Significant at the 1 percent level.
*Significant at the 5 percent level.
#Significant at the 10 percent level.

DROUGHT AND HEAT STUDIES
IN ALFALFA

by

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Drought and heat hardiness of alfalfa is of important under Kansas conditions. An investigation to study the reactions to drought and high temperatures in seven alfalfa varieties representing a wide range in growth characteristics and hardiness was conducted in 1960-61 at Manhattan, Kansas.

The investigation included the following experiments:

1. Germination, seedling growth and hardening is osmotic solutions of mannitol.
2. Effects of gibberellic acid on germination and seedling growth under different moisture tensions produced by mannitol.
3. Dry-matter yield and uptake of nitrogen, potassium, and phosphorus by plants grown in nutrient solutions having different moisture stresses.
4. Drought tests in flats filled with soil, sand, or vermiculite.
5. Emergence, growth, and total nitrogen content under different temperatures in growth chambers.
6. High-temperature tests at 130°F.
7. Dry-heat treatment of seed.
8. Effects of intra-variety selection for ability to germinate under high osmotic tension.

It was found that the ability to germinate under moisture stress produced by mannitol was heritable. This ability, however, was more closely related to vigor than to hardiness.

Seedlings of Teton, African, and Nomad were able to harden when germinated under six atmospheres moisture tension produced by mannitol while Buffalo, Ladak, Rhizoma, and Rambler seedlings did not harden.

On a relative basis, gibberellic acid promoted germination to a greater extent in the slower- than in the faster-growing varieties, and its stimulating effect on seedling growth increased with an increase in moisture stress.

Apparently, there was a progressive decrease in nitrogen, phosphorus, and potassium uptake from nutrient solution with an increase in moisture tension. Varietal differences in ability to absorb these nutrients under moisture stress were indicated.

Extreme variability and competition between plants indicated that the method of testing drought resistance in flats was unreliable.

In growth chambers, day temperature of 90°F. reduced seedling emergence and nitrogen content, but had no effect on dry weight, as compared with 70°F. Emergence and seedling growth were greatly reduced by 110°F.

Results of high-temperature tests suggested that temperature and relative humidity during tests must be controlled closely before reliable results can be obtained.

A high degree of relationship was found among the relative hardiness of the seven varieties in the embryo, seedling (hardened condition), and young-plant stages to three different types of injury (dry-heat at 194°F., plasmolysis in a 16 atmospheres mannitol solution followed by rapid deplasmolysis in water, and a moisture stress of 12 atmospheres, respectively). Results indicated significant differences in relative hardiness of varieties. The order of the seven varieties with respect to their hardiness was (highest to lowest): Teton, African, Buffalo, Nomad, Rambler, Ladak, and Rhizoma.